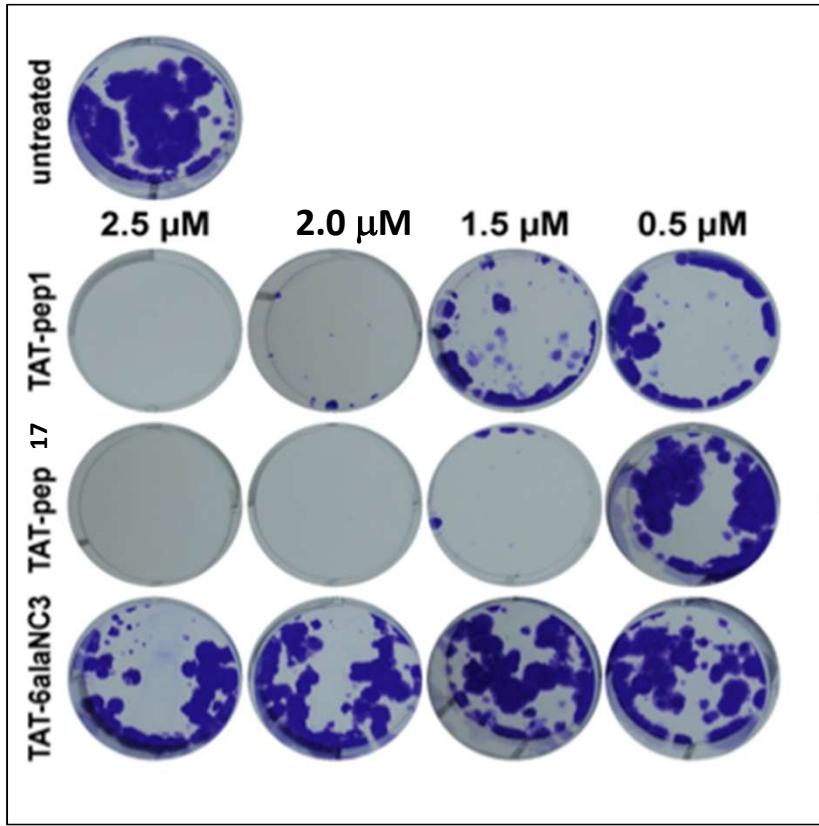


Design and Synthesis of Type IV Inhibitors of B-Raf Kinase that block dimerization and overcome paradoxical MEK/ERK activation

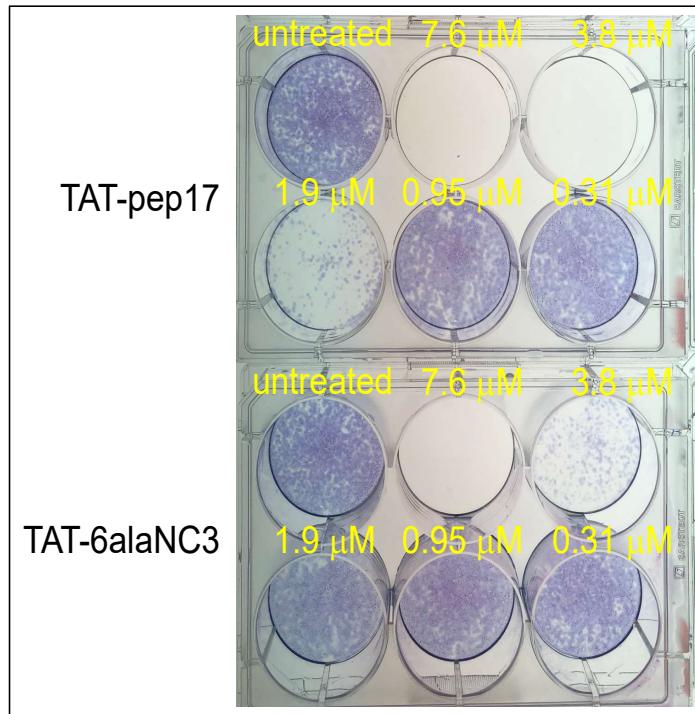
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Simone Galda ³, Sandra Brown³, Tilman Brummer^{3,4}, and Campbell McInnes^{1*}.

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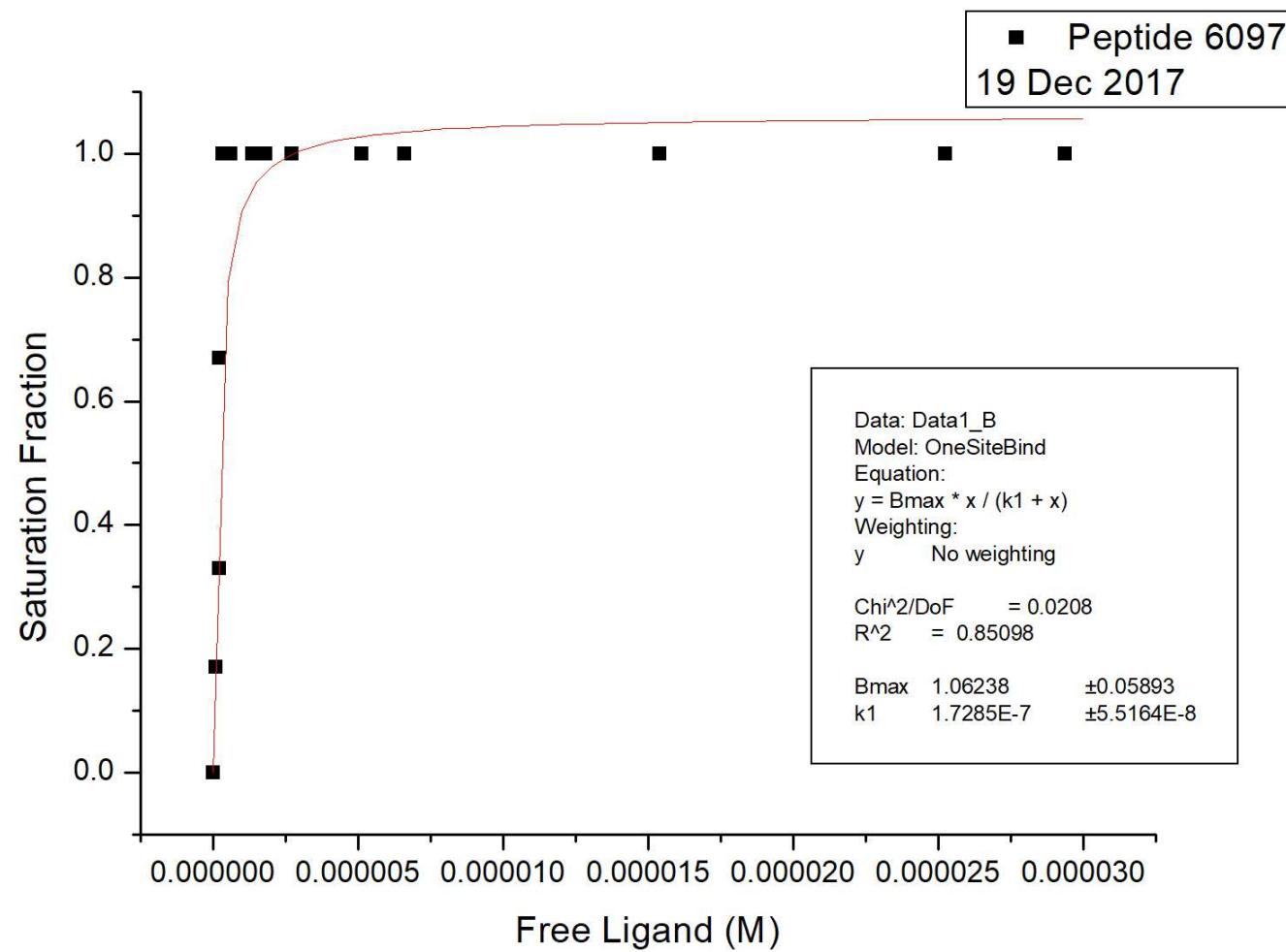
- S1 Colony formation assay using MCF-10A cells treated with TAT labelled peptides
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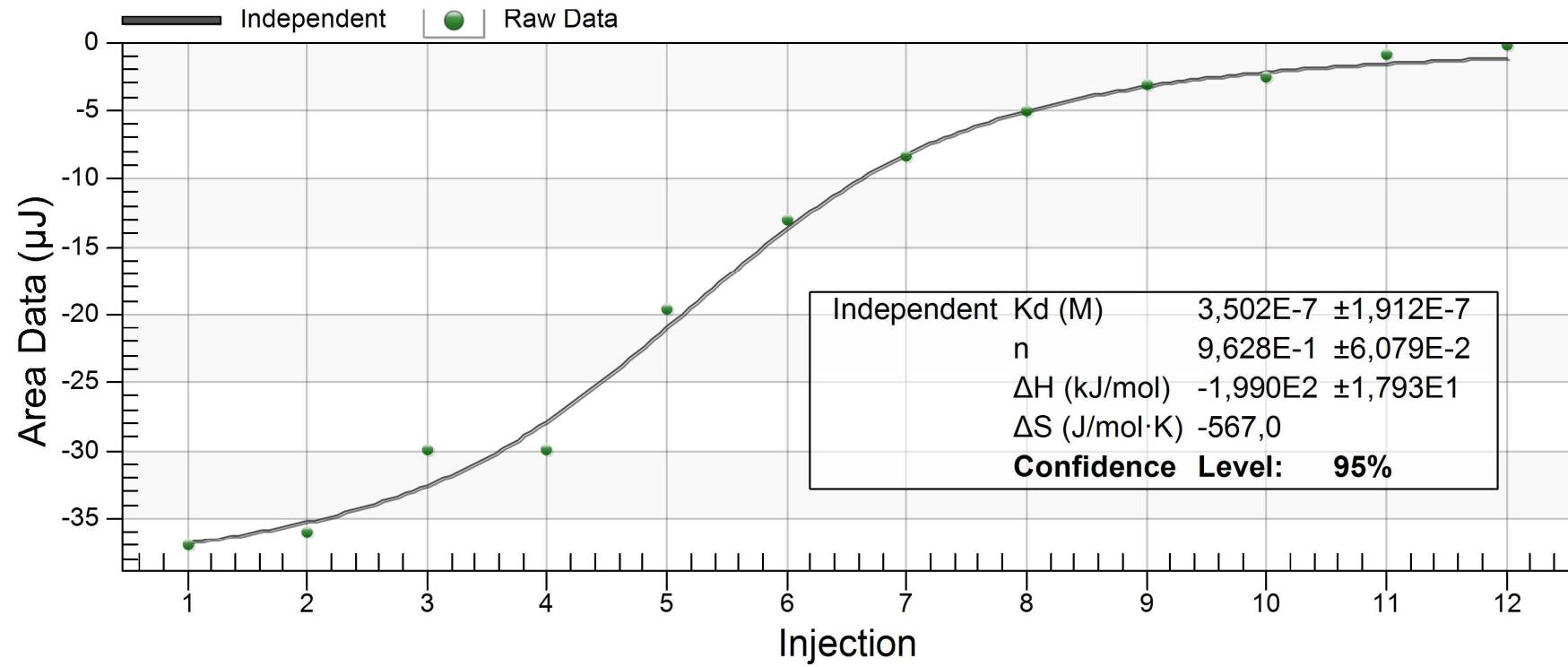
Supplementary Figure 1. Colony formation assay. 500 MCF-10A cells were plated onto 6 well dishes. Cells were grown for 11 days in presence of indicated peptide concentrations. Medium (containing peptides) was changed every second day. Peptides TAT-pep1 and TAT-pep13 impair colony growth in a dose dependent manner, while the inert TAT-6alaNC3 peptide does not affect proliferation.



Supplementary Figure 2. Unlabelled versions of the TAT peptides shown in Figure 2 yield comparable effects. Five thousand Sbcl2 cells were seeded onto 6-well plates and grown in the presence of the indicated peptide concentrations for two weeks. Medium with freshly added peptides were changed every 3 to 4 days. Cells were stained with Giemsa solution. Shown is a representative result from two independent biological replicates with comparable outcome



Supplementary Figure 3. Binding Curve obtained for Binding of Peptide 17 (6097) to BRAF using Intrinsic Trp Fluorescence



Supplementary Figure 4. Binding Curve obtained for Binding of Peptide 17 (6097) to BRAF using Isothermal Titration Calorimetry

Supplementary Table 1. Truncation Study of BRAF Dimer Interface Peptides

1	503-521	GVLRKTRHVNILLFMGYST	3.84 ±0.32
15	503-518	GVLRKTRHVNILLFMG	1.88 ±0.36
17	504-518	VLRKTRHVNILLFMG	0.13 ±0.040
31	505-518	LRKTRHVNILLFMG	0.19 ±0.13
16	504-517	VLRKTRHVNILLFM	5.75 ± 1.2

Supplementary Table 2. Analytical Data for PBD Binding Peptides

Peptide	Column Dimensions	Method	FlowRate	Retention Time	Theoretical MW	Observed MW
1	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	20.3	2205.6	2205.0
2	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	19.3	2185.5	2186.1
3	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	20.7	2205.6	2205.9
4	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.1	2163.5	2163.3
5	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	19.9	2178.6	2178.2
6	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	19.4	2162.6	2162.4
7	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	18.2	2219.6	2219.4
8	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.7	2175.6	2175.3
9	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	20.2	2215.7	2215.3
10	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	18.3	2177.6	2177.2
11	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.0	2163.6	2163.4
12	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	18.6	2205.6	2205.4
13	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	20.1	2219.6	2219.4
14	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.7	2173.6	2173.3
15	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.7	1854.3	1853.9
16	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.7	1740.2	1739.9
17	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.0	1797.2	1797.5
18	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	18.9	1796.2	1796.1
19	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	14.8	1839.3	1839.7
20	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	13.1	1781.2	1781.2
21	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	15.0	1755.2	1755.2
22	4.6 x 250 mm	5-95% acetonitrile/water/0.1%TFA/35 min	1ml/min	15.5	1712.1	1712.4
23	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	15.9	1740.1	1740.2
24	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	19.4	1712.1	1712.0
25	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	13.6	1731.2	1731.5
26	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.5	1754.2	1754.2
27	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.8	1755.2	1755.3
28	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	16.4	1755.2	1755.5
29	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	13.9	1721.1	1721.3
30	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	15.3	1737.1	1737.2
31	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	18.8	1698.1	1697.5
32	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/30 min	1ml/min	16.7	1655.1	1655.0
33	4.6 x 250 mm	5-65% acetonitrile/water/0.1%TFA/25 min	1ml/min	17.5	1684.1	1683.5
34	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/40 min	1ml/min	17.6	1683.1	1682.0
35	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/30 min	1ml/min	17.2	1707.0	1708.0
36	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/30 min	1ml/min	17.4	1664.0	1664.0
37	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/30 min	1ml/min	17.7	1722.1	1724.0
38	4.6 x 250 mm	5-95% acetonitrile/water/0.1%FA/30 min	1ml/min	18.2	1679.1	1680.0